CLAIMS

The claimed invention is:

1. A method for indexing double-stranded nucleic acid fragments from a mixture of double-stranded nucleic acid fragments, said method comprises the steps of:

(a) treating nucleic acids with one or more restriction endonucleases selected from the group consisting of Type IIS restriction endonuclease and interrupted palindrome (IP) restriction endonuclease to generate fragments having at least one 3'- or 5'-protruding single strand;

(b) incubating a C-indexer with said fragments generated in step (a) to allow selective hybridization between complementary protruding single strand of said fragments and said C-indexers, wherein said C-indexer is a double-stranded linear nucleic acid comprising a first strand and a second strand and a 3'- or a 5'-protruding single strand on said first strand and said second strand, wherein the lengths of the 3'- or 5'-protruding single strands of said C-indexer correspond to the lengths of the 3'- or 5'-protruding single strands of the fragments, wherein said C-indexer is selected from a collection of C-indexers whose 3'- or 5'-protruding single strands collectively encode up to all possible permutations and combinations of nucleotides, A, C, G and T;

(c) adding ligase to selectively ligate said C-indexer to those fragments whose 3'- or 5'-protruding single strands are fully complementary to the 3'- or 5'-protruding protruding single strands of said C-indexer; and

(d) obtaining circular indexed nucleic acid fragments, wherein the first strand of said C-indexer is ligated to the fragments to form a closed circular strand, and wherein the second strand of said C-indexer is ligated to the fragments to form a discontinuous strand.

2. The method of claim 1, wherein the length of the 3'- or 5'-protruding single strand of said C-indexer is longer than 2 bases.

3. The method of claim 2, the length of the 3'- or 5'-protruding single strand of said C-indexer is 3, 4, or 5 bases.

4. The method of claim 1, wherein the restriction endonuclease is a Type IIS restriction endonuclease.

5. The method of claim 4, wherein the Type IIS restriction endonuclease is selected from the group consisting of FokI, AarI, and HgaI.

6. The method of claim 1, wherein the restriction endonuclease is interrupted palindrome (IP) restriction endonuclease.

7. The method of claim 1, wherein the 5' end of the second strand of said C-indexer cannot be ligated.

8. The method of claim 1, wherein the second strand includes an affinity tag covalently attached to said C-indexer.

9. The method of claim 8, wherein the affinity tag is selected from a group consisting of a biotin moiety, a digoxigenin (DIG), a reactive amine, thiol group, an antibody, an antigen or oligonucleotide.

10. The method of claim 8, further comprising steps of:

(e) immobilizing said circular indexed nucleic acid fragments to a surface treated with an affinity target capable of binding said affinity tag through the affinity tag;

(f) washing away linear nucleic acid fragments;

(g) denaturing the closed circular strand and the discontinuous strand of the circular indexed fragments to release the closed circular strand from the solid surface;

(h) collecting the released closed circular strand of the circular indexed fragments.

11. The method of claim 10, wherein the surface is a plastic surface, a glass surface or a membrane surface.

12. A method of amplifying circular indexed nucleic acid fragments, said method comprises the steps of:

(a) treating nucleic acids with one or more restriction endonucleases selected from the group consisting of Type IIS restriction endonuclease and interrupted palindrome (IP) restriction endonuclease to generate fragments having at least one 3'- or 5'-protruding single strand;

(b) incubating a C-indexer with said fragments generated in step (a) to allow selective hybridization between complementary protruding single strand of said fragments and said C-

indexers, wherein said C-indexer is a double-stranded linear nucleic acid comprising a first strand and a second strand and a 3'- or a 5'-protruding single strand on said first strand and said second strand, wherein the lengths of the 3'- or 5'-protruding single strands of said C-indexer correspond to the lengths of the 3'- or 5'-protruding single strands of the fragments, wherein said C-indexer is selected from a collection of C-indexers whose 3'- or 5'-protruding single strands collectively encode up to all possible permutations and combinations of nucleotides, A, C, G and T;

(c) adding ligase to selectively ligate said C-indexer to those fragments whose 3'- or 5'-protruding single strands are fully complementary to the 3'- or 5'-protruding protruding single strands of said C-indexer;

(d) obtaining circular indexed nucleic acid fragments, wherein the first strand of said C-indexer is ligated to the fragments to form a closed circular strand, and wherein the second strand of said C-indexer is ligated to the fragments to form a discontinuous strand; and

(e) amplifying the closed strand of said circular indexed nucleic acid fragments by rolling circle amplification.

13. The method of claim 12, wherein the length of the 3'- or 5'-protruding single strand of said C-indexer is longer than 2 bases.

14. The method of claim 13, the length of the 3'- or 5'-protruding single strand of said C-indexer is 3, 4, or 5 bases.

15. The method of claim 12, wherein the restriction endonuclease is a Type IIS restriction endonuclease.

16. The method of claim 15, wherein the Type IIS restriction endonuclease is selected from the group consisting of FokI, AarI, and HgaI.

17. The method of claim 12, wherein the restriction endonuclease is interrupted palindrome (IP) restriction endonuclease.

18. The method of claim 12, wherein the 5' end of the second strand of said C-indexer cannot be ligated.

19. The method of claim 12, wherein said discontinuous strand is used as rolling circle amplification primer.

20. The method of claim 12, wherein the second strand of said C-indexer is covalently attached to an affinity tag.

21. The method of claim 20, wherein the affinity tag is selected from a group consisting of a biotin moiety, a digoxigenin (DIG), a reactive amine, thiol group, an antibody, an antigen or oligonucleotide.

22. The method of claim 20, wherein the circular indexed nucleic acid fragments are immobilized to a surface treated with an affinity target capable of binding said affinity tag through said affinity tag before said rolling circle amplification.

23. The method of claim 22, wherein the surface is plastic surface, glass surface or membrane surface

24. The method of claim 22, wherein said rolling circle amplification is performed on the surface.

25. The method of claim 22, wherein said rolling circle amplification is performed after said closed circular strand is separated from said discontinuous strand.

26. A kit for indexing double-stranded nucleic acid fragments from a mixture of double-stranded nucleic acid fragments, said kit comprising: (a) one or more Type IIS or IP restriction endonucleases; (b) a C-indexer, wherein said C-indexer is a double-stranded linear nucleic acid comprising a first strand and a second strand and a 3'- or a 5'-protruding single strand on said first strand and said second strand, wherein the lengths of the 3'- or 5'-protruding single strands of said C-indexer correspond to the lengths of the 3'- or 5'-protruding single strands of nucleic acid fragments generated by Type IIS or IP restriction endonucleases digestion, wherein said C-indexer is selected from a collection of C-indexers whose 3'- or 5'-protruding single strands collectively encode up to all possible permutations and combinations of nucleotides, A, C, G and T; and (c) ligase and ligase buffer.

27. The kit of claim 26, wherein the 5' end of the second strand of said C-indexer is not phosphorylated.

28. The kit of claim 26, wherein the second strand of said C-indexer is covalently attached to an affinity tag.

29. The kit of claim 28, further comprising a surface treated with an affinity target capable of binding said affinity tag.

30. The kit of claim 26, further comprising a DNA polymerase, a mixture of all four deoxynucleotide precursors, and a rolling circle amplification buffer.

31. A method for indexing single-stranded nucleic acid fragments from a mixture of single-stranded nucleic acid fragments, said method comprises the steps of:

(a) incubating a C-indexer with single-stranded nucleic acid fragments to allow selective hybridization between complementary protruding single strands of said C-indexer with the 5' end and 3' end of said single-stranded nucleic acid fragments; wherein said C-indexer is a double-stranded linear nucleic acid comprising a first strand and a second strand and a 5'- and a 3'-protruding single strand on said second strand, wherein said C-indexer is selected from a collection of C-indexers whose 5'- and 3'-protruding single strands collectively encode up to all possible permutations and combinations of nucleotides, A, C, G and T;

(b) adding ligase to selectively ligate said C-indexer with those single-stranded nucleic acid fragments whose 5'- and 3'-ends are fully complementary, respectively, to the 5'- and 3'-protruding single strands of said C-indexer; and

(c) obtaining circular indexed nucleic acid fragments, wherein the first strand of said C-indexer is ligated to the fragments to form a closed circular strand, wherein the second strand of the C-indexer is not closed.

32. The method of claim 31, wherein the lengths of the 5'- and 3'-protruding single strand on the second strand of said C-indexer are the same.

33. The method of claim 31, wherein the lengths of the 5'- and 3'-protruding single strand on the second strand of said C-indexer are different.

34. The method of claim 31, wherein the lengths of the 5'- and 3'-protruding single strand on the second strand of said C-indexer are between 3 and 30.

35. The method of claim 31, wherein the 5' end of the second strand of said C-indexer cannot be ligated.

36. The method of claim 31, wherein the second strand includes an affinity tag covalently attached to said C-indexer.

37. The method of claim 36, wherein the affinity tag is selected from a group consisting of a biotin moiety, a digoxigenin (DIG), a reactive amine, a thiol group, an antibody, an antigen or oligonucleotide.

38. The method of claim 31, wherein the single-stranded nucleic acids can be generated from denaturing double-stranded nucleic acids.

39. A method of amplifying circular indexed single-stranded nucleic acid fragments, said method comprises the steps of:

(a) incubating a C-indexer with single-stranded nucleic acid fragments to allow selective hybridization between complementary protruding single strands of said C-indexer with the 5' end and 3' end of said single-stranded nucleic acid fragments; wherein said C-indexer is a double-stranded linear nucleic acid comprising a first strand and a second strand and a 5'- and a 3'-protruding single strand on said second strand, wherein said C-indexer is selected from a collection of C-indexers whose 5'- and 3'-protruding single strands collectively encode up to all possible permutations and combinations of nucleotides, A, C, G and T;

(b) adding ligase to selectively ligate the first strand of said C-indexer with those single-stranded nucleic acid fragments whose 5'- and 3'-ends are fully complementary, respectively, to the 5'- and 3'-protruding single strands of said C-indexer;

(c) obtaining circular indexed nucleic acid fragments, wherein the first strand of said C-indexer is ligated to the nucleic acid fragments to form a closed circular strand, wherein the second strand of the C-indexer is not closed; and

(d) amplifying the closed strand of said circular indexed nucleic acid fragments by rolling circle amplification.

40. The method of claim 39, wherein the lengths of the 5'- and 3'-protruding single strand on the second strand of said C-indexer are the same.

41. The method of claim 39, wherein the lengths of the 5'- and 3'-protruding single strand on the second strand of said C-indexer are different.

42. The method of claim 39, wherein the lengths of the 5'- and 3'-protruding single strand on the second strand of said C-indexer are between 3 and 30.

43. The method of claim 39, wherein the 5' end of the second strand of said C-indexer cannot be ligated.

44. The method of claim 39, wherein the single-stranded nucleic acids can be generated from denaturing double-stranded nucleic acids.

45. The method of claim 39, wherein said second strand of the C-indexer is used as rolling circle amplification primer.

46. The method of claim 39, wherein the second strand of said C-indexer is covalently attached to an affinity tag.

47. The method of claim 46, wherein the affinity tag is selected from a group consisting of a biotin moiety, a digoxigenin (DIG), a reactive amine, a thiol group, an antibody, an antigen or oligonucleotide.

48. The method of claim 46, wherein the circular indexed nucleic acid fragments are immobilized to a surface treated with an affinity target capable of binding to said affinity tag through said affinity tag before said rolling circle amplification.

49. The method of claim 48, wherein said surface is a plastic surface, glass surface or membrane surface

50. The method of claim 48, wherein said rolling circle amplification is performed on the surface.

51. The method of claim 48, wherein said rolling circle amplification is performed after said closed circular strand is separated from said second strand of the C-indexer.

52. A kit for indexing single-stranded nucleic acid fragments from a mixture of single-stranded nucleic acid fragments, said kit comprising: (a) a C-indexer, wherein said C-indexer is a double-stranded linear nucleic acid comprising a first strand and a second strand and a 5'- and a 3'-protruding single strand on said second strand, wherein said C-indexer is selected from a collection of C-indexers whose 5'- and 3'-protruding single strands collectively encode up to all possible permutations and combinations of nucleotides, A, C, G and T; and (b) ligase and ligase buffer.

53. The kit of claim 52, wherein the 5' end of the second strand of said C-indexer is not phosphorylated.

54. The kit of claim 52, further comprising a nucleic acid polymerase, a mixture of nucleotide precursors, and a buffer for rolling circle amplification reaction.

55. The kit of claim 52, wherein one of the strands of said C-indexer is covalently attached to an affinity tag.

56. The kit of claim 55, wherein the affinity tag is selected from a group consisting of a biotin moiety, a digoxigenin (DIG), a reactive amine, a thiol group, an antibody, an antigen or oligonucleotide.

57. The kit of claim 56, further comprising a surface treated with an affinity target capable of binding said affinity tag.

58. The kit of claim 52, wherein the single-stranded nucleic acids can be generated by denaturing double-stranded nucleic acids.